Locally Advanced Intrahepatic Cholangiocarcinoma Successfully Resected after Transcatheter Arterial Chemoembolization with Degradable Starch Microspheres: Report of a Case

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SUMMARY

We describe a case of initially unresectable locally advanced intrahepatic cholangiocarcinoma that showed remarkable regression after transcatheter arterial chemoembolization with degradable starch microspheres, allowing for subsequent successful curative resection. A 75-year-old female was referred to our hospital with a large hepatic mass. Computerized tomography examination showed a huge mass in the right liver extended partially to the left liver. Intrahepatic cholangiocarcinoma was strongly suspected, but surgical resection was abandoned due to the local spread in the liver. Three courses of transcatheter arterial chemoembolization with degradable starch microspheres were performed. The anticancer agents, mitomycin C and epirubicin, combined with degradable starch microspheres were injected from the catheter for chemoembolization. After three courses of transcatheter arterial chemoembolization, the tumor size decreased from 10cm to 5.5cm in diameter. Then right trisegmentectomy together with extra-hepatic bile duct excision was performed. At 25 months after the first therapy and 21 months after operation, the patient remains healthy without recurrence. Transcatheter arterial chemotherapy with degradable starch microspheres may be a treatment of choice with locally advanced intrahepatic cholangiocarcinoma.

KEY WORDS:
Degradable starch Microspheres; Intrahepatic cholangiocarcinoma; Liver resection

ABBREVIATIONS:
Intrahepatic Cholangiocarcinoma (ICC); Degradable Starch Microspheres (DSM); Transcatheter Arterial Chemoembolization (TACE)

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) accounts for 3-5% of all primary liver malignancies (1,2). The incidence is increasing and its pathogenesis remains unclear (3-5). Currently, complete surgical resection with histologically negative margins is the only cure for this formidable entity and yields a five-year survival of 13-42% (2,6). By contrast, in patients not electing surgical therapy, the prognosis is extremely poor, with a median survival of less than 1 year (7,8). Unfortunately, the overall resectability rate of ICC is unsatisfactory, ranging from 19-70% (2,9). Noncurative operation offers no survival benefit, with a median survival of only approximately 3 months (10). Thus, increasing the rate of curative resection is of vital importance for patients with ICC.

In this paper, we report a case of initially unresectable locally advanced ICC that showed remarkable

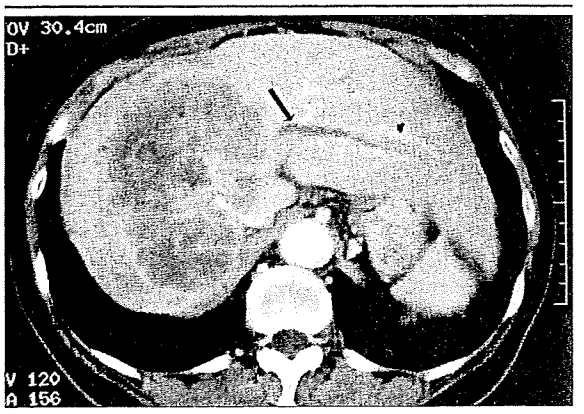

FIGURE 1 A 75-year-old female with cholangiocarcinoma. Intravenous contrast-enhanced CT scan of the upper abdomen demonstrating a huge solid mass, 10.0x9.0cm in size with peripheral enhanced areas in the right lobe of the liver. Involvement of the umbilical portion and slight dilation of the bile duct in Segment 2 is visible (arrow).

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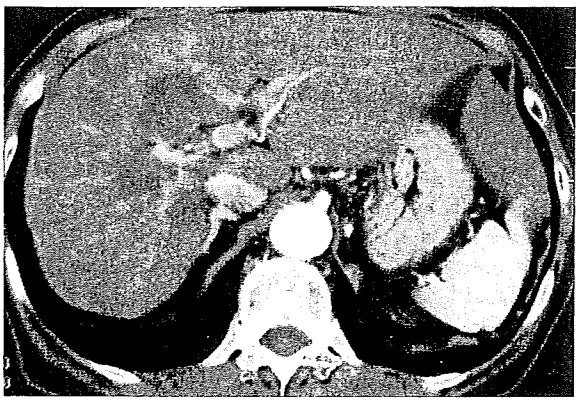

FIGURE 2 Intravenous contrast-enhanced CT scan showing lipiodol accumulation in the tumor and decreased tumor size 1 week after the third chemoembolization.

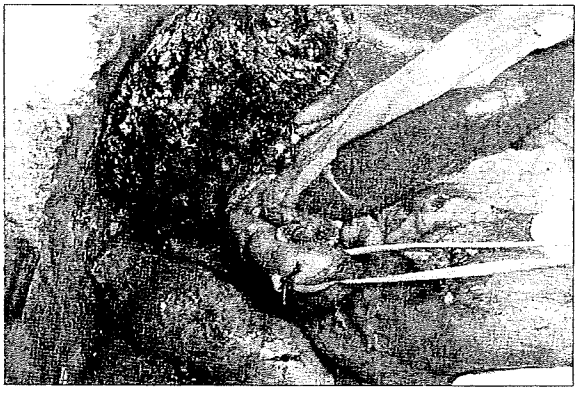

FIGURE 3 Raw surface of the liver after right trisegmentectomy of the liver together with the extra-hepatic bile duct excision. The surgical margin was free from cancer.

regression after transcatheter arterial chemoembolization with degradable starch microspheres (DSM-TACE), allowing for subsequent successful curative resection.

CASE REPORT

A 75-year-old female was referred to our hospital with right-sided abdominal pain, liver dysfunction, and a large hepatic mass. Computerized tomography (CT) examination showed a solid mass 10cm in diameter with peripheral enhancement in the right liver. The tumor extended partially to the left liver, adjacent to the umbilical portion of the liver. CT scan also disclosed slight dilation of the bile duct in the left lateral segment of the liver **(Figure 1)**. Gastrointestinal studies revealed no other abnormal findings. Serum alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and CA19-9 were elevated at 1286 U/L, 2774 U/L, and 430.7 U/mL, respectively. ICC was strongly suspected, but surgical resection was deferred secondary to local spread in the liver.

Three courses of transcatheter arterial chemoembolization with degradable starch microspheres (DSM-TACE) were performed. On December 17, 2003, the first DSM-TACE treatment was undertaken. Arteriography of the hepatic artery was performed to characterize the vascular supply of the lesion. The anticancer agents, mitomycin C (6mg) and epirubicin (60mg), combined with DSM (600mg), were injected from the catheter for chemoembolization. The right hepatic artery and left hepatic artery were embolized at a ratio of 3:1. On January 26, 2004, DSM-TACE was repeated using the same protocol with different doses of the chemo-embolic agents (DSM, 300mg; epirubicin, 50mg; mitomycin C, 6mg). The third course was performed on February 17, 2004, again using DSM (450mg), mitomycin C (6mg) and epirubicin (55mg) for chemoembolization. In this last treatment, the artery supplying the left medial segment of the liver was embolized. No major complications or side effects were noted during or after each DSM-TACE session.

After three courses of DSM-TACE, the serum level of CA19-9 decreased from 430.7 U/mL to 25.8 U/mL, and the tumor size decreased from 10cm to 5.5cm in diameter **(Figure 2)**. Surgical resection was then elected. Right trisegmentectomy together with extrahepatic bile duct excision was performed on April 16, 2004 **(Figure 3)**. Postoperative pathology confirmed a margin negative resection and a moderately differentiated intrahepatic cholangiocarcinoma without extrahepatic lymphatic metastasis **(Figure 4)**.

Postoperative recovery was excellent without complications. At 21 months after the first DSM-TACE therapy and 25 months after operation, the patient remains healthy and tumor free with normal serum tumor marker levels.

DISCUSSION

Treatments for unresectable ICC include chemotherapy, radiotherapy, photodynamic therapy, radiofrequency thermal ablation, and liver transplantation (11). Although some investigators have reported an improved prognosis following such treatment, the benefits tend to be limited, with poor life expectancy, and outcomes have not been verified via prospective and randomized clinical studies. Thus, improving the resectability rate of these tumors is of great significance for patient outcomes.

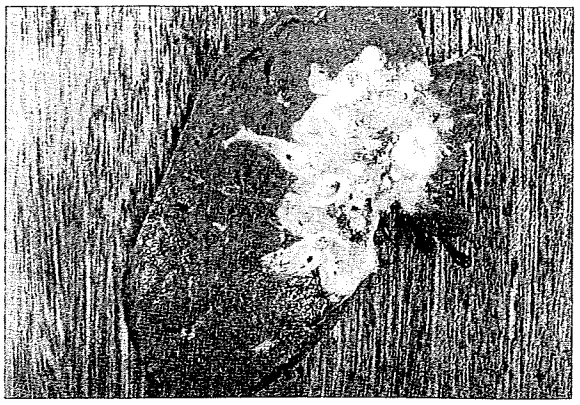

FIGURE 4 Macroscopic findings of the resected specimen. A 7.5x5.0-cm intrahepatic cholangiocarcinoma was located in the hepatic hilum with an adequate surgical margin.

In the present case, a margin-negative resection was initially considered impossible due to the local advancement to the umbilical portion of the liver. Degradable starch microspheres (DSM) are 40-45 microns in diameter and can be degraded by serum amylase within 30 to 60 minutes. When a mixture of DSM and chemotherapeutic agents are applied via transcatheter arterial infusion, transient occlusion of the arterial blood flow can enhance regional retention of the anti-tumor agents and reduce systematic toxicity. Furthermore, transient vascular occlusion induced by DSM can lead to the redistribution of blood flow to hypovascular areas and thereby potentiate the chemotherapeutic effects on ICC, which is typically a hypovascular tumor (12). Moreover, repeated administrations are possible, and DSM-TACE is well tolerated even in cases with portal vein trunk obstruction.

The use of DSM-TACE for treatment of unresectable hepatocellular carcinoma (HCC) and metastatic liver diseases is increasing secondary to improved patient outcomes. For example, the response rate is 52.9-72.7% for patients with HCC, 42.8% for patients with metastatic gastric cancer, and 37.8-70% for patients with colon cancer, with complete responses observed in some cases (13-16). However, there are comparatively few published reports regarding the efficacy of DSM-TACE in patients with unresectable ICC. In 1989, Hirai *et al.* described a series of 11 cases of HCC and one case of ICC treated by DSM-TACE. While 72.7% of the HCC cases experienced a decrease in tumor size in response to treatment, there was no notable change in the case with ICC (14). By contrast, in the present report, tumor size decreased by 70% after DSM-TACE, thereby enabling curative resection.

In conclusion, unresectable ICC is associated with extremely poor prognosis, however, DSM-TACE may be of utility in such cases in establishing local control, improving quality of life, downstaging the disease, and even facilitating curative resection in some cases. DSM-TACE is considered to be a treatment of choice in patients with locally advanced ICC.

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CT and MRI Findings with Contrast Enhancement of Small Pancreatic Adenocarcinoma in the Late Phase

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SUMMARY
A 60-year-old female was found to have high serum amylase concentrations at a medical check-up. Dynamic computed tomography and magnetic resonance imaging demonstrated a mass in the body of the pancreas, which was enhanced in the late phase of the scans by administration of a contrast medium. Endoscopic retrograde pancreatography showed a stenosis of the main pancreatic duct at the body, and brushing cytology from the region revealed adenocarcinoma. Distal pancreatectomy was performed. The tumor was a well-differentiated adenocarcinoma, measuring 15x10mm. Fibrous tissues were sparsely distributed in the tumor, and there was an increase of dilated veins, in particular at the margin. Late-phase enhancement of the tumor with computed tomography or magnetic resonance imaging was considered to be correlated with this abundant vascular structure in the tumor. Marked tumor enhancement in the late phase might be a characteristic finding suggesting an early-stage pancreatic adenocarcinoma, which should be carefully checked.

KEY WORDS:
Pancreatic carcinoma; Computed tomography; Pancreatic neoplasm; Late phase

ABBREVIATIONS:
Computed Tomography (CT); Magnetic Resonance Imaging (MRI); Endoscopic Retrograde Pancreatography (ERP); Ultrasonography (US); Multidetector Computed Tomography (MDCT)

INTRODUCTION

Despite the advent of sophisticated imaging modalities, a majority of pancreatic ductal adenocarcinomas are diagnosed at stages IVA or IVB. The five-year survival rate of pancreatic ductal adenocarcinomas is still less than 5% (1,2), and the median survival time for unresectable cases ranges from 4 to 8 months. For a better prognosis, a tumor should be detected at an early stage and excised curatively.

In pancreatic adenocarcinoma, which is usually hypovascular, computed tomography (CT) shows a low-attenuation mass in the early pancreatic phase after infusion of the contrast medium. Magnetic resonance imaging (MRI) demonstrates similar findings. However, few detailed reports have been published concerning CT or MRI findings in small pancreatic adenocarcinomas. Our case demonstrated a marked enhancement of a pancreatic tumor in the late phase. Late-phase enhancement of small pancreatic carcinoma has not been studied previously in terms of radiological and pathological correlation. We present here a case of small pancreatic adenocarcinoma for which we studied the mechanism of marked late-phase enhancement. Description of the tumor extent is made based on the sixth edition of the TNM Classification (UICC) (3).

CASE REPORT

A 60-year-old female was found to have high amylase blood concentrations at a medical check-up in April 2004 and visited a clinic. Abdominal ultrasonography (US) and CT revealed dilatation of the main pancreatic duct at the pancreatic body and tail, and she was referred to our hospital. Biochemistry showed no abnormality, except high levels of serum and urinary amylase. Tumor markers were all within the normal range, except elastase I.

US at 3.5MHz frequency demonstrated a tumor measuring 9x8mm at the pancreatic body with dilatation of the pancreatic duct in the tail **(Figure 1a)**. US at 7.5MHz revealed that the tumor had an ill-defined

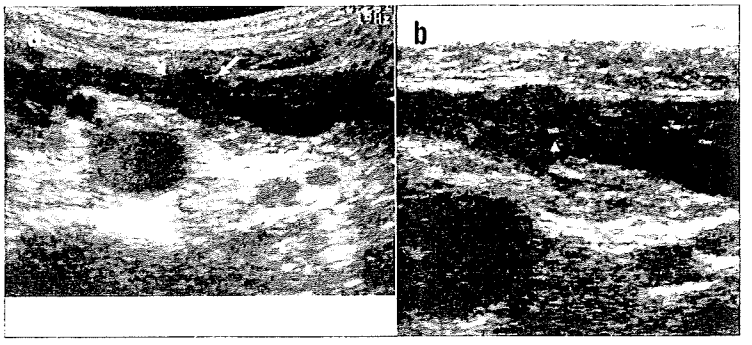

FIGURE 1 US at 3.5MHz frequency **(a)** demonstrated a low-echoic mass measuring 9x8mm at the pancreatic body (arrow) with dilatation of the pancreatic duct in the tail. US at 7.5MHz **(b)** revealed that the tumor had an ill-defined margin and high echoic spots (arrowhead), probably reflecting a duct structure within the mass.

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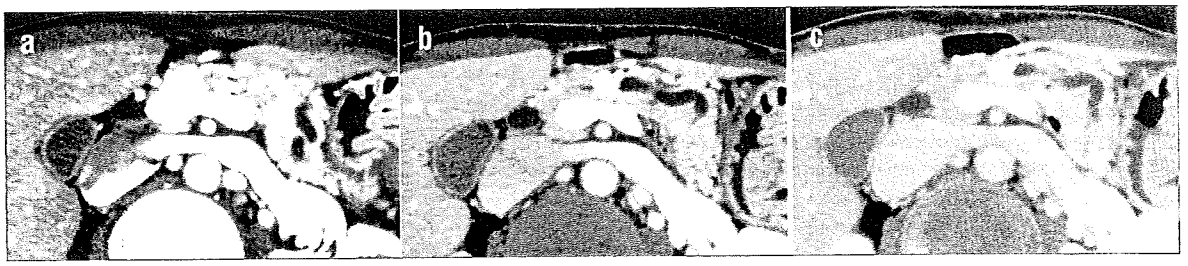

FIGURE 2 Multidetector CT shows a hypoenhancing mass at the pancreatic phase 40 sec after contrast administration **(a)**. However, it showed homogeneous contrast enhancement at the portal phase, 80 sec after contrast administration **(b)**, and the enhancement lasted until the late phase, 180 sec after contrast administration **(c)**.

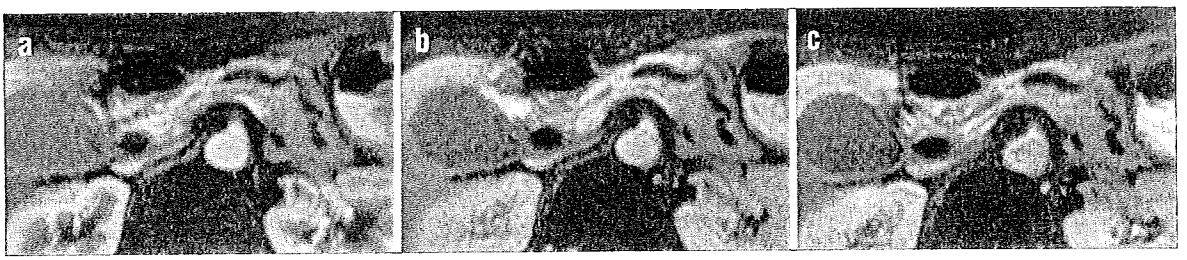

FIGURE 3 Dynamic MRI did not show the tumor 20 sec after infusion of the contrast medium **(a)**, but the whole tumor was revealed by contrast enhancement 80 sec after the infusion **(b)**, and the enhancement lasted until 180 sec **(c)**.

margin and an isogenic internal echo with high echoic spots (**Figure 1b**). Multidetector CT (MDCT) with four detectors was performed using 100mL of a contrast medium at a concentration of 370mg/dL. The contrast medium was intravenously infused at a rate of 3mL/sec. Scans were started at 40, 80 and 180 sec after initiation of contrast administration. Images were taken at 1.25-mm collimation and reconstructed with a width of 0.5mm. The tumor was visualized as a hypo-enhancing mass (162HU) at the pancreatic phase 40 sec after contrast administration (**Figure 2a**), but it showed homogeneous contrast enhancement (204HU) at the portal phase, 80 sec after contrast administration, and the enhancement lasted (164HU) until the late phase, 180 sec after contrast administration (**Figure 2c**).

In MRI, both T1- and T2-weighted images demonstrated dilatation of the pancreatic duct, but did not show a tumor. Dynamic MRI was performed using gadrinium DTPA; the tumor was not visualized 20 sec after infusion of the contrast medium (**Figure 3a**), but the whole tumor was revealed by contrast enhancement 80 sec after the infusion (**Figure 3b**), and the enhancement lasted until 180 sec (**Figure 3c**).

Endoscopic retrograde pancreatography (ERP) showed a stricture of the main pancreatic duct at the pancreatic body, and brushing cytology from the region revealed atypical cells characterized by enlargement, size heterogeneity, increased chromatin content of the nuclei, enlargement of nucleoli, and mucus in the cytoplasm, leading to the diagnosis of adenocarcinoma. Although the mass was enhanced in the late phase of both CT and MRI, the tumor was diagnosed as a resectable pancreatic adenocarcinoma and distal pancreatectomy was performed.

Cut surfaces of resected specimens revealed that the tumor was slightly whitish and ill demarcated (**Figure 4a**). The tumor was 15x10mm in size. The low-magnified view is shown in **Figure 4b**. There was exfoliation of the epithelium in the main pancreatic duct, possibly due to the postoperative catheterization of the duct by a thin catheter, which was performed to obtain a pancreatogram for comparison with ERP. A histological examination showed a well-differentiated adenocarcinoma infiltrating the pancreatic parenchyma with tubular and glandular formations (**Figure 4c**). Fibrous tissues were sparsely distributed in the stroma. In addition, there was an increase in markedly dilated veins in the tumor, in particular at the margin of the tumor (**Figure 4d**).

The final pathological diagnosis was as follows: Invasive ductal adenocarcinoma of the pancreas; well differentiated tubular adenocarcinoma, T1, intermediate type, INF β, ly1, v0, N0 Stage I.

DISCUSSION

Despite the advent of sophisticated imaging methods, the five-year survival rate for patients with ductal adenocarcinomas has been less than 5% for decades (1,2), and even in resected cases the rate has been approximately 10~20% (4-8). The prognosis of pancreatic adenocarcinomas is poorer among malignant tumors because they are likely to extend beyond pancreatic ductal walls into parenchyma and invade vascular and lymphatic structures or perineural spaces. In pancreas, there is no barrier, such as muscle layer around pancreatic ductal walls, which probably could facilitate cancer invasion at an earlier stage (9-13). However, patients with respectable small carcinoma less than 2cm in size have the favorable survival of 30-40% at five years (8,14), and the diagnosis of as small

carcinoma as possible should be made for better prognosis.

CT and MRI are imaging modalities useful for the diagnosis of pancreatic cancer. So far, CT and MRI have been effective methods to stage pancreatic adenocarcinoma, but not for detection of small pancreatic tumors because these methods provide poorer resolution than does US. However, recent development of multi-detector CT remarkably has improved spatial resolution, and it has become possible to diagnose small pancreatic tumors.

Since ductal adenocarcinoma of the pancreas shows a poorly demarcated cut surface, hard consistency with fibrous stroma and poor vascular structures histologically, it is visualized by dynamic CT in the pancreatic phase as a hypoenhancing mass relative to the normal pancreas (15), 30-40 sec after administration of contrast medium, when the normal parenchyma is best enhanced. In the late phase, the contrast medium begins to wash out of the normal tissue and infiltrate into the fibrous stroma of the tumor, thus providing mild enhancement.

Lu, Vedantham, Boland, Furukawa *et al.* reported that visualization of pancreatic adenocarcinoma was significantly higher in the pancreatic phase, when the tumor-pancreas contrast difference was maximal, than in the portal phase (15-18). In general practice, detection of a low-attenuation area in the pancreatic phase is thought to be an important sign in the diagnosis of pancreatic adenocarcinoma.

However, in the present case, dynamic CT in the pancreatic phase not only demonstrated a slightly lower attenuation area than the adjacent pancreatic parenchyma, but showed remarkable tumor staining in the late phase. MRI showed similar late-phase enhancement of the tumor. As mentioned previously, tumor staining in the late phase is thought to derive from the abundant fibrous stroma of the pancreatic adenocarcinoma, but pathological findings in the present case revealed mild fibrosis with abundant fibroblasts and a limited number of hyalinized collagen fibers. Although venous invasion was negative, the number of dilated veins was increased, particularly at the tumor margin. Although the mechanism underlying the fibrosis of pancreatic adenocarcinomas has not been understood fully, it can be hypothesized that after an early stage of fibrosis, which possibly is associated with an increased number of dilated veins, stenosis occurs in these veins in association with hyalinized fibrotic changes and tumor infiltration.

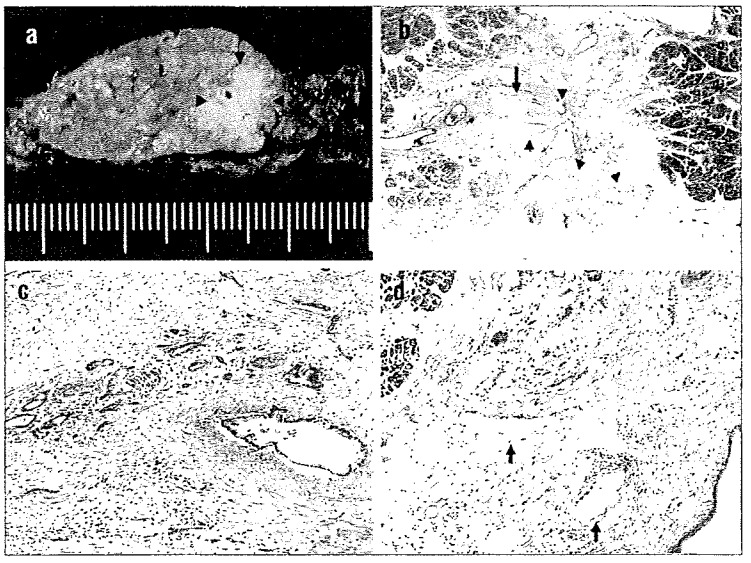

FIGURE 4 Cut surfaces of resected specimens revealed a slightly whitish and ill demarcated tumor. The tumor was 15x10mm in size **(a)**. The low-magnified view **(b)** revealed the main pancreatic duct (arrow) and branch ducts (arrowheads). A histological examination showed a well-differentiated adenocarcinoma infiltrating the pancreatic parenchyma with tubular and glandular formations **(c)**. There was an increase in the number of dilated veins (arrows) in the tumor, particularly at the margin of the tumor **(d)**.

In the present case, CT and MRI demonstrated tumor enhancement in the late phase, which seemed to reflect an increased number of dilated veins, as is often recognized in hemangioma, and the remarkable tumor staining in the late phase might be a characteristic CT or MRI finding suggestive of relatively early-stage pancreatic adenocarcinomas. Indeed, Yamaguchi *et al.* (19) reported that tumor staining was observed in the late phase by CT in 70% of pancreatic cancer 20mm or smaller in diameter, compared with 20% of pancreatic tumors 21mm or larger. Therefore, it is important to pay attention to tumor staining in the late phase, and not to overlook a low-attenuation area in the pancreatic phase, for diagnosis of small pancreatic cancer.

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ORIGINAL ARTICLE

Effects of tumor selective replication-competent herpes viruses in combination with gemcitabine on pancreatic cancer

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Abstract
Purpose Pancreatic cancer still has a poor prognosis, even if aggressive therapy is pursued. Currently, new modalities of oncolytic virus therapy are being tested against this cancer. The combination of one of two representative mutant herpes simplex viruses (R3616: $\gamma_1$34.5 inactivated, hrR3: UL39 inactivated) with a standard anti-pancreatic cancer chemotherapy drug (gemcitabine), was investigated in this study.
Experimental design The intracellular concentration of ribonucleotide reductase was estimated by Western blotting. The effect of gemcitabine on viral replication and the total cytotoxic effect of the combination therapy were investigated on pancreatic cancer cell lines. We compared the results of two oncolytic viruses, R3616 and hrR3. A mouse model of pancreatic cancer with peritoneal dissemination was used to evaluate the in vivo effect of the combination therapy.
Results Although the replication of both viruses was inhibited by gemcitabine, the combination caused more tumor cell cytotoxicity than did virus alone in vitro. The results with R3616 were more striking. Although the difference was not statistically significant, R3616 with gemcitabine had a greater effect than did R3616 alone, while hrR3 with gemcitabine had a weaker effect than did hrR3 alone in vivo experiments.
Conclusion The combination of oncolytic virus with gemcitabine is a promising new strategy against advanced pancreatic cancer. Each virus has different functional characteristics, and can affect the results of the combination of viruses and chemotherapy drugs. The results indicate that there is a complicated interaction among viruses, cells, and chemotherapy drugs and that the best combination of oncolytic virus and chemotherapeutic agents should be studied more extensively before embarking on a clinical trial.

Keywords Herpes oncolytic virus · Pancreatic cancer · Gemcitabine · Combination therapy

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Introduction

Pancreatic cancer is a disease with an extremely poor prognosis. Surgical therapy for pancreatic cancer is still insufficient to cure most patients [1]. Recently gemcitabine has shown a modest survival advantage over 5-fluorouracil (5-FU) in patients with this cancer [2]. Gemcitabine has become one of the standard chemotherapy drugs against pancreatic cancer but more effective therapies must be devised in order to significantly improve survival. Oncolytic virus therapy has been highly trusted as a new type of therapy for advanced incurable pancreatic cancer, and may provide some clinical benefit to those patients in the near future. Currently, clinical trials using oncolytic viruses have been started against many types of cancer in world-wide [3], such as brain cancer [4, 5], prostate cancer [6, 7], pancreatic cancer [8], breast cancer [9], and head and neck cancer [10, 11]. This study investigated the possibility of combination therapy using gemcitabine and two herpes mutant oncolytic viruses (R3616 and hrR3) against pancreatic cancer.

Gemcitabine (difluorodeoxycytidine; dFdC) is intracellularly phosphorylated to difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate

(dFdCTP). dFdCTP competes with deoxycytidine triphosphate (dCTP) for incorporation into DNA, and DNA synthesis is inhibited [2, 12, 13]. In addition, dFdCDP acts as an inhibitor of ribonucleotide reductase (RR) in cells, which in turn causes a major decrease in the dCTP pool. Therefore, gemcitabine reduces the activity of RR in cancer cell lines [14] (Fig. 1a). However, some cells have been known to acquire chemoresistance to gemcitabine due to over expression of RR [15–21].

R3616 and hrR3 are genetically engineered herpes simplex viruses [3]. R3616 lacks the $\gamma_1$34.5 gene that produces the ICP34.5 protein. Replication of R3616 is severely restricted in normal cells, because the expression of ICP34.5 in normal cells prevent a protein shutoff mechanism that is associated with eIF2α dephosphorylation through the protein kinase receptor (PKR). Most cancer cells lose this normal protein shutoff mechanism so that viral replication can proceed, which induces the virally infected cells to undergo apoptosis to protect the integrity of the cell's DNA and block viral replication [3, 22–24]. hrR3 lacks the UL39 gene that produces the ICP6 proteins (viral RR), a key enzyme in the biosynthesis of DNA in all prokaryotic and eukaryotic cells. The viral replication of hrR3 is severely restricted in cells that have high levels of holding proteins involved in nucleic acid synthesis such as cancer cells [25, 26] (Fig. 1b).

We investigated the effect of tumor-selective, replication-competent herpes viruses (R3616 and hrR3) against pancreatic cancer under the same conditions in which gemcitabine effects cancer cells. Our major concern was how gemcitabine may interrupt viral replication, and whether the combination of an oncolytic virus with gemcitabine can significantly improve anti-pancreatic cancer therapy.

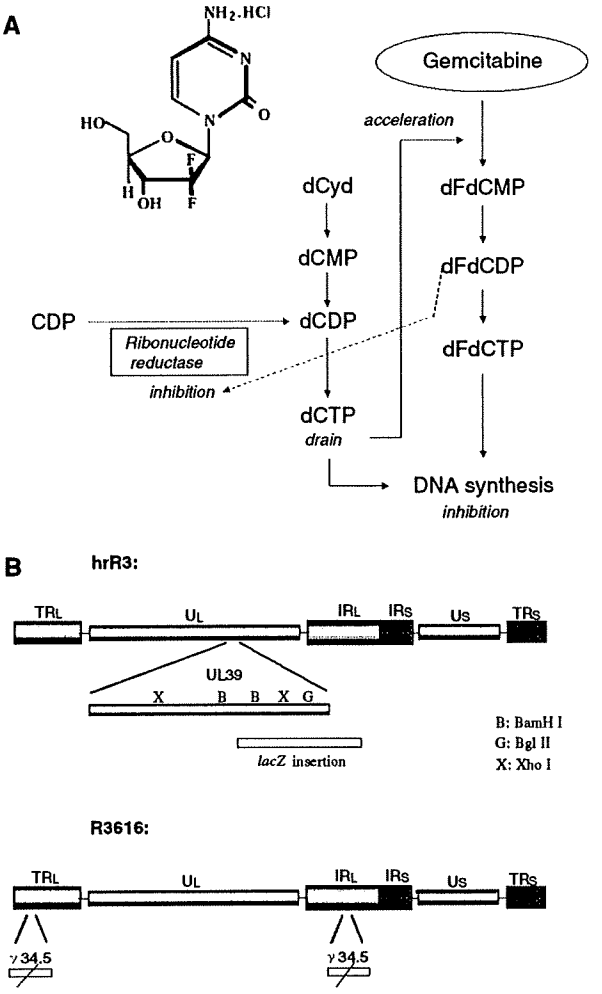

Fig. 1 **a** Gemcitabine structure and pathway. Gemcitabine HCl is a nucleoside analog that exhibits anti-tumor activity. Gemcitabine HCl is 2′-deoxy-2′, 2′-difluorocytidine monohydrochloride (β-isomer). The empirical formula for gemcitabine HCl is C9H11F2N3O4 × HCl. It has a molecular weight of 299.66. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. **b** Schematic illustration of hrR3 and R3616. hrR3 is a mutated herpes simplex virus (*HSV*) that has the LacZ gene inserted into the site of UL39 (ICP6), causing inactivation of ribonucleotide reductase activity that is associated with UL39. Ribonucleotide reductase is a key enzyme for viral DNA synthesis. R3616 is a mutated HSV that has a deletion of both $\gamma_1$34.5 genes. The $\gamma_1$34.5 gene produces ICP 34.5 that dephosphorylates eIF2α-phosphate to permit continued viral protein synthesis. Those mutated HSVs replicate and destroy only the cancer cells

Materials and methods

Viruses and cells

R3616 was kindly provided by Bernard Roizman Sc. D (University of Chicago, Chicago, IL, USA) and hrR3 was kindly provided by Sandra K. Weller Ph.D. (University of Connecticut, Storrs, CT, USA). SW1990, derived from a human pancreatic carcinoma, was kindly provided by Dr. T. Sawada (First Department of Surgery, Osaka City University, Osaka, Japan). CAPAN 1, also derived from a human pancreatic carcinoma, was obtained from the Japanese Cancer Research Resources Bank, Tokyo, Japan. PACA2, another cell line derived from a human pancreatic carcinoma, was obtained from the American Type Culture Collection, Manassas, VA, USA. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and 1% penicillin/streptomycin at 37°C (Sigma, Tokyo, Japan).

Western blot assay

A total of 10^6 cells were harvested and rinsed twice with phosphate-buffered saline, pH 7.4. Cell extracts were prepared with lysis buffer (20 mM Tris, pH 7.5, 0.1% Triton-X, 0.5% deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) and clarified by

centrifugation at 12,000*g*, for 15 min, at 4°C. Cell lysates containing equal amounts of protein as determined by a BCA assay kit were electrophoresed on a NuPAGE, Novex 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, USA), and the resolved proteins were transferred to PVDF membranes (Invitrogen).The membranes were blocked with 5% nonfat milk overnight at room temperature, and incubated with 0.2 μg/ml human anti-RRM1 antibody (CHEMICON International, Temecula, CA, USA) for 1 h. RRM1 was detected using an enhanced chemiluminescence (ECL) system following the manufacturer's instructions (Amersham Life Science, Uppsala, Sweden). β-actin also was detected on the same membrane to serve as a control for the amount of protein loaded.

Cytotoxic assay

Gemcitabine and viral-induced cytotoxicity assays were performed using the MTT assay as previously described [27, 28]. Briefly, 10^6 cells were plated in a 10-cm plate and 10 μg/ml of gemcitabine was added. After 24 h, a replication-competent virus (R3616 or hrR3) was added at multiplicity of infection (MOI) values ranging from 0.01 to 10 and incubated for an additional 48 h. The number of surviving cells was quantified by a colorimetric MTT assay. The results, expressed as mean ± SD of four samples, were compared with the results from the cytotoxicity assays of gemcitabine alone and the virus alone. Statistical significance was determined by the two-sided Student's *t*-test using SPSS (SPSS, Chicago, IL, USA).

Viral replication assay

Viral replication assays were performed as described [28, 29]. Briefly, 10^6 cells were plated in a 10-cm plate and 10 μg/ml of gemcitabine was added. After 24 h, replication competent viruses (R3616 or hrR3) were added at MOI of 2. Forty-eight hours after infection, the supernatant and cells were harvested, exposed to three freeze- thaw cycles to release the virions, and titered. The results were compared with the assays of viral replication without gemcitabine.

Animal studies

Mice (6-week-old females BALB/c nu/nu) were obtained from the Charles River Japan, Yokohama, Japan. Animal studies were performed in accordance with the guidelines issued by the Nagoya University Animal Center. The mice, used in a peritoneal-disseminated carcinoma model, were injected with 10^6 PACA2 cells into the intraperitoneal cavity. The condition of the animals was checked once or twice a day for the duration of the study. The mice were divided randomly into six groups (A–F). Group A ($n = 10$), group D ($n = 10$), and group E ($n = 10$) were injected with 1 mg of gemcitabine into the intraperitoneal cavity on day 14 after the injection of the PACA2 cells. The mice in groups A and B ($n = 10$) each were injected with 10^6 particles of R3616 on day 15 after the injection of the PACA2 cells. Group C ($n = 10$) and group D ($n = 10$) were injected with 10^6 particles of hrR3 on day 15 after the injection of the PACA2 cells. Group F ($n = 10$) was the control group, which was injected with only PACA2 cells into the intraperitoneal cavity.

Statistical differences between groups were determined by the log-rank test with the use of JMP 5.0 software (SAS Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Expression of RRM1 by Western blotting

As previously reported by many researchers on their papers, overexpression of ribonucleotide reductase subunit 1 (RRM1) is associated with chemoresistance to gemcitabine [15–17]. We examined the intensity of RRM1 protein expression in Capan1, PACA2, and SW1990 cells (Fig. 2). The intensity of RRM1 expression in the PACA2 cells was greater than in the other cell lines. The results from many previous related papers regarding chemoresistance to gemcitabine, indicated that PACA2 cells might have the highest potential of chemo resistance to gemcitabine among the three cell lines.

Comparison of cytotoxic assays between hrR3 and R3616, with or without gemcitabine

We compared the cytotoxicity of R3616 ($\gamma_1$34.5 deficiency) and hrR3 (ICP6: RR gene deficiency) viruses' combination with gemcitabine by the MTT assay (Fig. 3). With both R3616 and hrR3, the cytotoxicity was increased by their

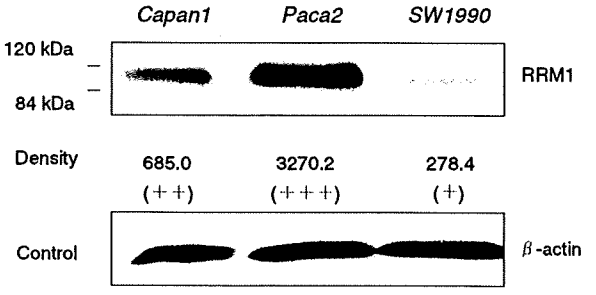

Fig. 2 Expression of ribonucleotide reductase M1 (*RRM1*) by Western blotting. PACA2 cells expressed the most ribonucleotide reductase M1 (*RRM1*) by Western blot assays among three pancreatic cancer cell lines tested. β-actin served as a control for the amount of protein loaded in each lane

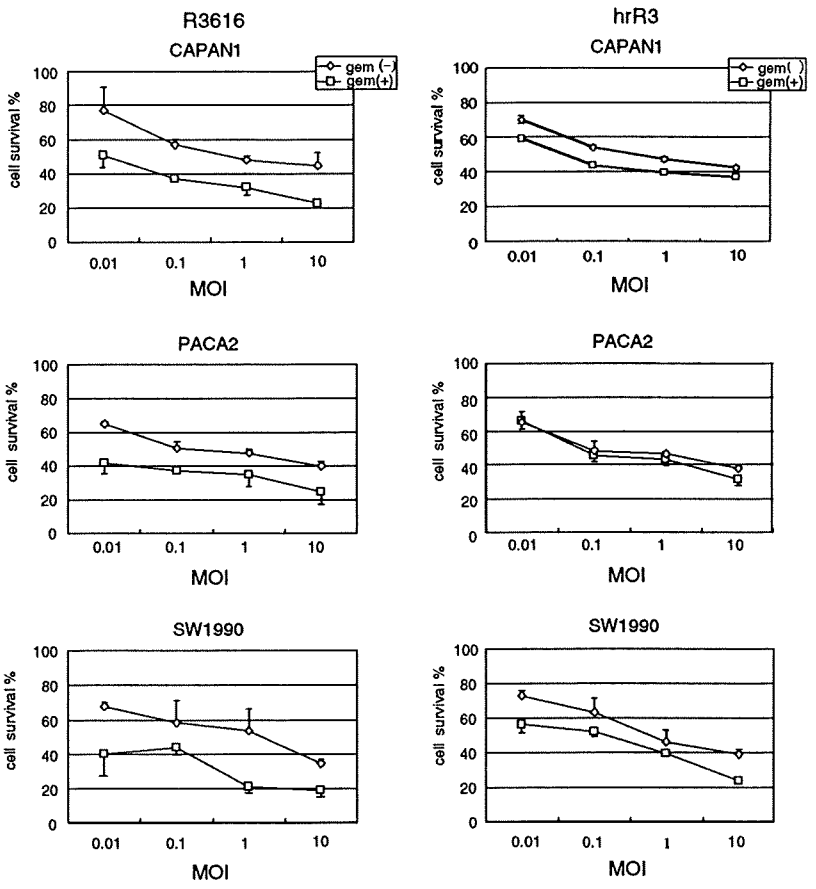

Fig. 3 Comparison of cytotoxic assays between hrR3 and R3616, with or without gemcitabine. For both R3616 ($\gamma_1$34.5 gene inactivated) and hrR3 (ICP6: ribonucleotide reductase gene inactivated), the cytotoxity was increased when combined with gemcitabine. However, there was a trend toward greater cytotoxity with R3616 than with hrR3 against all cell lines

combination with gemcitabine, but the more significant increase in cytotoxicity was observed with R3616 than hrR3. On the other hand, PACA2 cells, which expressed the most RRM1 by a Western blot assay, had the lowest increase in cytotoxicity with the combination of hrR3 and gemcitabine.

Comparison of cytotoxic assays between gemcitabine alone and gemcitabine with low titer virus

We also compared the cytotoxicity between gemcitabine alone and gemcitabine with low titer virus by the MTT assay (Fig. 4). Of all cell lines, the combination of gemcitabine and an MOI 0.01 of R3616 showed more cytotoxic tendency than did gemcitabine alone ($P = 0.04$o nP ACA2 cell line), while the combination of gemcitabine with an MOI 0.01 of hrR3 tend to be less cytotoxic than gemcitabine alone. PACA2 cells.

Comparison of viral replication between hrR3 and R3616, with or without gemcitabine

We compared the viral replication between R3616 and hrR3 in the presence of gemcitabine by the plaque-forming assay (Fig. 5). The replication of both viruses was inhibited by gemcitabine. The titer of hrR3 declined more than did R3616 in combination with gemcitabine. The replication of hrR3 was inhibited by gemcitabine in all cell lines. PACA2 cells expressed the most RRM1 by Western blot assay, and hrR3 replicated more vigorously with gemcitabine in the PACA2 cells than in the other two cell lines, while R3616 was also inhibited by gemcitabine in all cell lines but with somewhat weaker inhibition comparing to hrR3.

Animal studies

Long-term survival (LTS: 100 days) was achieved in 60% of mice treated with an intraperitoneal injection of R3616 followed by gemcitabine (group A). Mice treated with an intraperitoneal injection of R3616 had only a 50% LTS (group B). Mice treated with hrR3 had a 30% LTS (group C). Mice treated with hrR3 followed by gemcitabine had a 20% LTS (group D). Mice treated with gemcitabine alone had only a 10% LTS (group E). All mice in the control group died within 60 days (group F) (Fig. 6). Statistical differences in the survival rates were determined by log-rank analyses (group A versus group F, $P = 0.0011$; group A versus group D, $P = 0.0078$; group E versus group F,

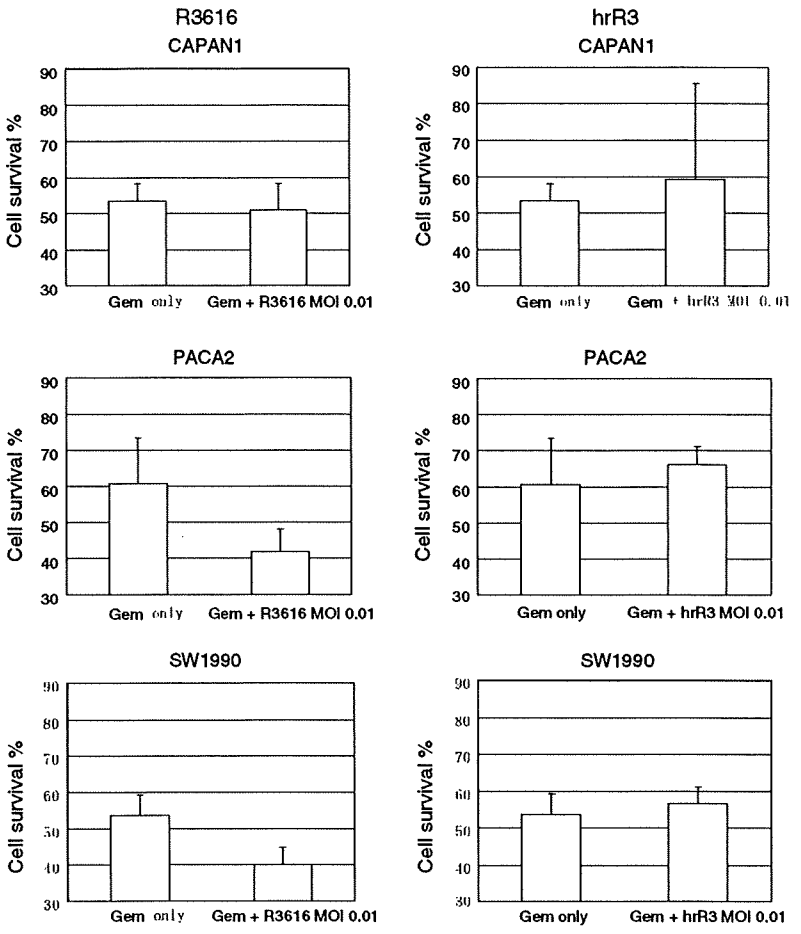

Fig. 4 Comparison of cytotoxic assays between gemcitabine alone and gemcitabine with a very low titer of virus. For all cell lines, the combination of gemcitabine and a very low titer (MOI 0.01) of R3616 tended to exhibit greater cytotoxicity than did gemcitabine alone ($P = 0.04$ on PACA2 cell line), on the other hand, the combination of gemcitabine and a very low titer (MOI 0.01) of hrR3 tended to be less cytotoxic than gemcitabine alone

$P = 0.006$; group B versus group D, $P = 0.0174$; group B versus group F, $P = 0.0049$). There were no other statistically significant differences between the other groups except for shown above. Although it was not significantly different, R3616 with gemcitabine tended to have a stronger effect than did R3616 alone, while hrR3 with gemcitabine tended to be weaker than hrR3 alone.

Discussion

In this study, we compared the efficacy of hrR3 or R3616 plus gemcitabine against pancreatic cancer. An in vitro cytotoxic assay indicated that R3616 plus gemcitabine caused a significant increase in the cell-killing effect in all three pancreatic cancer cell lines than did hrR3. We postulate that this result was due to the functional differences caused by each deleted viral gene. The viral replication of hrR3 might be more interrupted by the effect of gemcitabine than that of R3616, and this reduction might have been responsible for the slight decrease in the cell-killing effect of hrR3. Cellular RR is important for viral replication especially for hrR3 that has no RR [3, 25–27, 29, 30]. Gemcitabine is well known to reduce the activity of cellular RR in cancer cell lines [14]. Therefore, it is a possible that the effect of gemcitabine was greater in combination with hrR3 than with R3616 reducing the replication and cytotoxicity of the viruses.

Interestingly, infection with hrR3 at a very low concentration (MOI 0.01) in the presence of gemcitabine caused less cytotoxic than did gemcitabine alone. This may be the result of the virus protecting the cancer cells from the apoptosis caused by gemcitabine. The virus itself has some anti-apoptotic effects on cells in order to protect the host cells from bursting too early and until the virus particles have matured. Although gemcitabine reduced the replication of hrR3, some viral anti-apoptosis genes might still have worked in the infected cells without the burst-cell effect that is caused by an abundance of mature viruses. The apoptosis mechanism might malfunction as a result of this low virus concentration, causing an anti-apoptotic effect against gemcitabine. This effect might apply not only to HSV, a critical

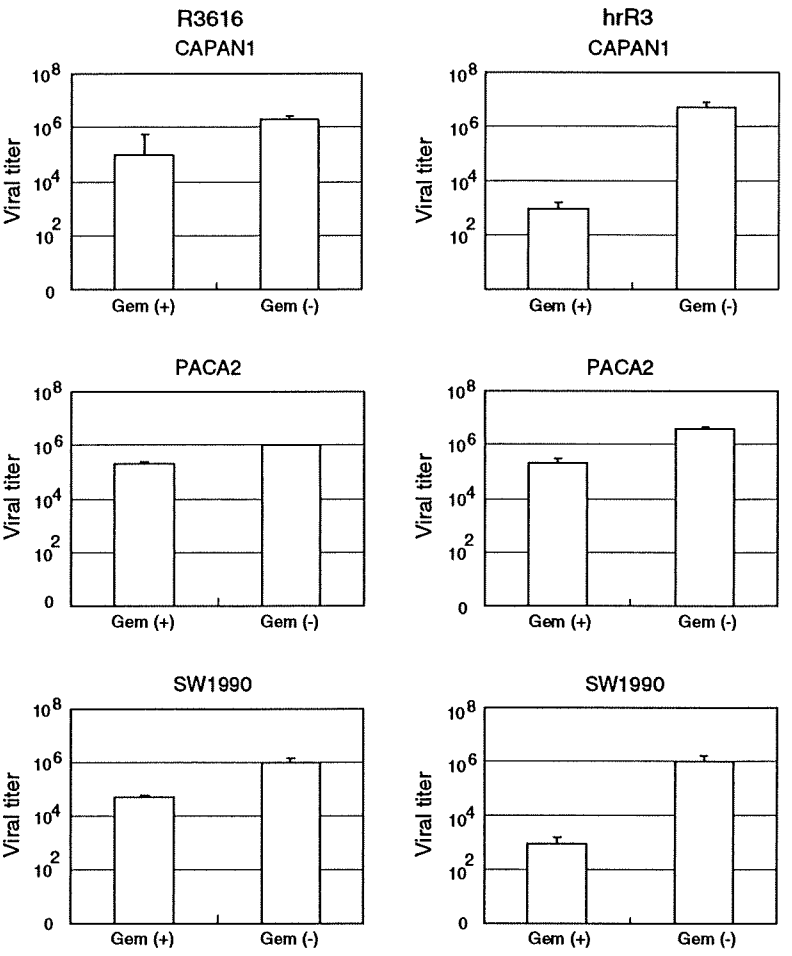

Fig. 5 Comparison of viral replication between hrR3 and R3616, with or without gemcitabine. The viral replication of both R3616 and hrR3 were inhibited by the presence of gemcitabine. This phenomenon was more prominent with hrR3 and gemcitabine than with R3616

consideration when using a viral vector or an oncolytic virus with chemotherapy drugs, because most viruses have such an anti-apoptosis gene. Examples include US3 and US5 in HSV [31, 32], and E1b 19 kDa in adenovirus [33]. Furthermore, several distinct viruses have been shown to develop mechanisms to block premature apoptosis of infected cells [34–36]. This phenomenon should be considered when using any viral vector for gene therapy or oncolytic virus therapy with chemotherapy drugs. In our opinion, the anti-apoptosis genes in a virus should be studied more intensively if future development of oncolytic virus therapy is to proceed.

PACA2 cells had the highest density of RR by Western assays and also the lowest cytotoxic effect from single agent gemcitabine among the three pancreatic cancer cell lines tested as 60% cell survival in Fig. 4, which indicates that PACA2 cells have some type of resistance against the cytotoxicity of gemcitabine comparing to other two cell lines. For the combination of R3616 with gemcitabine, increased efficacy was observed against all the pancreatic cancer cell lines even if the cells had some resistance to the chemotherapy alone. On the other hand, the combination of hrR3 with gemcitabine was of weak cytotoxity toward PACA2 cells, which expressed the most RRM1 by Western blot assay, and the effect was less pronounced than when R3616 was used. These results suggest that, the combination of R3616 and gemcitabine might be suitable for the cancer cell type that is expected to offer resistance to gemcitabine.

In the in vivo experiments, the combination of R3616 with gemcitabine yielded a 60% LTS rate (100 days) in the mice. This was higher than in mice treated with an intraperitoneal injection of R3616 alone that resulted in a 50% LTS rate, while mice treated with only hrR3 had a 30% LTS rate; however, there was no statistically significant difference in the LTS rate between R3616 and R3616 with gemcitabine. Thus, combination therapy with R3616 and gemcitabine had the same or slightly higher efficacy than the virus alone. However, mice treated with hrR3 followed by gemcitabine showed a lower LTS rate (20%) than those treated with hrR3 alone. And moreover, there was a statistically significant difference between group A (R3616 + GEM) and group D (hrR3 + GEM) ($P = 0.0078$). From the results of our in vivo and in vitro, we determined that the

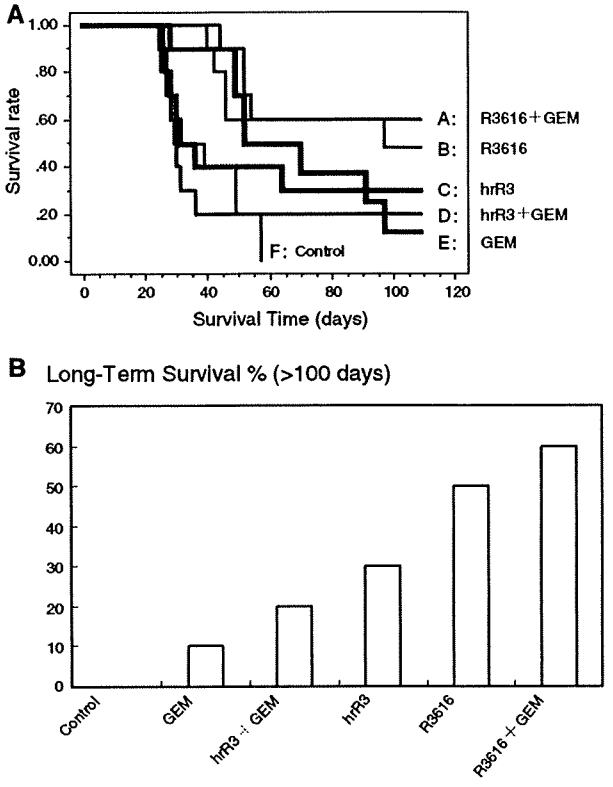

Fig. 6 **a** Cumulative survival curves of an in vivo mouse model. The PACA2 cells were injected into the peritoneal cavity. Each group was treated as shown below. Group A: R3616 + gemcitabine (GEM), group B: R3616 only, group C: hrR3 only, group D: hrR3 + GEM, group E: GEM only, and group F: no treatment. Differences in the survival rates were assessed by log-rank analysis (group A versus group F, $P = 0.0011$; group A versus group D, $P = 0.0078$; group E versus group F, $P = 0.006$; group B versus group D, $P = 0.0174$; and group B versus group F, $P = 0.0049$). There were no other statistic ally significant differences between the other groups except for shown above. There was a statistically significant difference between group A (R3616 + GEM) and group D (hrR3 + GEM) ($P = 0.0078$). **b** Long-term survival (*LTS*: over 100 days). LTS was achieved in 60% of mice treated with an intraperitoneal injection of R3616 followed by gemcitabine (group A). Mice treated with an intraperitoneal injection of R3616 only had a 50% LTS survival rate (group B). Mice treated with hrR3 only had a 30% LTS rate (group C). Mice treated with hrR3 followed by gemcitabine had a 20% LTS rate (group D). Mice treated with gemcitabine alone had only a 10% LTS rate (group E). The untreated group (group F) had a 0% LTS survival rate

combination of gemcitabine with R3616 ($\gamma_1$34.5 inactivated) might be more effective than the combination with hrR3 (RR inactivated).

Potentially, chemotherapy drugs connote to inhibit oncolytic virus replication to some degree, but this effect may be influenced by the differences in the characteristics of each virus caused by gene mutation. UL 39 (ICP6)-deleted HSVs, such as G207 [3, 37], and Myb34.5 [38, 39] also have some kind of potential likely to be inhibited by gemecitabine, as is hrR3, because of the genetic characteristics of RR. In other words, UL39-intact HSVs, such as HF10 [40, 41], RH105 [42], and DFγ34.5 [43] are likely to interact differently from a UL 39-deleted HSV (e.g., hrR3), in combination therapy with gemcitabine. Additional studies must be needed for further confirmation of the efficacy depending upon the functional characteristics among the chemotherapy drugs, viruses, and the cancer cells.

In the future, oncolytic virus therapy in combination with chemotherapy drugs may become more popular for use in clinical trials. Therefore, the characteristics of each virus must be considered carefully to determine if they are suitable for use with the chemotherapy drugs chosen.

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Original Paper

Analysis of Clinicopathological Features and Predictors of Malignancy in Intraductal Papillary Mucinous Neoplasms of the Pancreas

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KEY WORDS:
IPMN; IPMC; predictor; PHRSD

ABBREVIATIONS:
Intraductal Papillary Mucinous Neoplasm (IPMN); Mucinous Cystic Neoplasm (MCN); World Health Organization (WHO); Intraductal Papillary Mucinous Carcinoma (IPMC); Computed Tomography (CT); Magnetic Resonance Cholangiopancreatography (MRCP); Endoscopic Retrograde Cholangiopancreatography (ERCP); Endoscopic Ultrasonography (EUS); Main Pancreatic Duct (MPD); Carcinoma *In Situ* (CIS); Carbohydrate Antigen 19-9 (CA19-9); Carcinoembryonic Antigen (CEA); Pancreaticoduodenectomy (PD); Pylorus-preserving Pancreaticoduodenectomy (PpPD); Pancreatic Head Resection with Segmental Duodenectomy (PHRSD); Segmental Resection of the Pancreatic Body (SR);

ABSTRACT

Background/Aims: Intraductal papillary mucinous neoplasms (IPMNs) are increasingly recognized, but it is very difficult to evaluate accurately the malignancy of these neoplasms by modern imaging. We reviewed our experience in order to elucidate predictors of tumor malignancy, invasiveness, and outcome.

Methodology: The clinicopathological features and surgical outcomes of 57 patients with IPMNs who underwent surgery in Nagoya University Hospital were analyzed.

Results: The histological diagnosis was adenoma in 40, borderline in 1, carcinoma *in situ* (CIS) in 7, and invasive carcinoma in 9 patients. Patients with invasive carcinomas had significantly shorter survival rates than patients with benign IPMNs or CIS ($p<0.0001$). Multivariate analyses revealed that the main duct or the combined type was significantly predictive of malignancy, and both main duct or combined type and diabetes mellitus were associated significantly with invasive carcinoma.

Conclusions: IPMNs generally grow slowly, but have a malignant potential that warrants radical surgical treatment when the tumor component invades the parenchyma. Our results suggest that the above factors should be considered in surgical management. The main duct type of IPMN or IPMN with mural nodules is potentially malignant or invasive. Therefore, radical operative management is indicated in these IPMNs.

INTRODUCTION

Intraductal papillary mucinous neoplasms (IPMNs) were described in the Japanese literature in 1982 (1) and in the English literature in 1986 (2). This entity is increasingly recognized and is characterized by an adenomatous proliferation of pancreatic duct epithelium that may involve the main pancreatic duct or ductal branches alone or in combination (3,4). IPMN is comprised of a spectrum of diseases from benign to malignant (5,6). This neoplasm is rare, constituting only 1% of pancreatic cancers, according to previous reports (7,8). Before the concept of IPMNs was established, confusion with mucinous cystic neoplasms (MCNs) or other cystic tumors of the pancreas existed (9). In 1996, the World Health Organization (WHO) established criteria to classify IPMNs and to distinguish them from MCNs (10). IPMNs exhibit different stages from adenoma with mild atypia to invasive carcinoma (10), but lack the ovarian stroma characteristics of MCNs. Most IPMNs, including non-invasive intraductal papillary mucinous carcinomas (IPMCs), have a more favorable prognosis than pancreatic ductal carcinoma, however, some cases of invasive IPMC have a poor prognosis (11-15). Therefore, appropriate management requires the differentiation of malignant tumors, but it is very difficult to evaluate accurately the extent of malignancy by modern imaging.

In this study, we retrospectively reviewed our experience and analyzed the clinicopathological features of IPMNs to elucidate predictive factors associated with malignancy, invasiveness, and tumor outcome.

METHODOLOGY

Fifty-seven patients who underwent surgery for IPMNs between March 1991 and July 2004 in Nagoya University Hospital were reviewed. We retrospectively examined the clinicopathological features, surgical outcomes, and factors predictive of malignancy. The study was approved by the Ethics Committee of the hospital. Informed consent was obtained from all patients for the subsequent use of resected tissues. All patients underwent radiographic and endoscopic examination such as computed tomography (CT), mag-

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netic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), and endoscopic ultrasonography (EUS). The tumors were classified into three subtypes based on the principal site of tumor involvement as follows: main duct type, branch duct type, and combined type (both main duct and branch duct involved). The diameter of the main pancreatic duct (MPD) was measured by ERCP. The size of the tumor was measured by ultrasonography or EUS.

All pathologic specimens were reviewed by a pathologist (T.N.) in order to confirm the diagnosis of IPMN. They were classified as IPM adenoma, borderline IPMN, carcinoma *in situ* (CIS), and invasive IPMC, according to the criteria established by the WHO. Tumors that featured minimal stromal invasion were classified as invasive carcinoma. We divided our cases into three groups: benign IPMN, CIS, and invasive IPMC. The benign IPMN group included adenomas and borderline tumors. The malignant group included CIS and invasive IPMC. Patient data including age, gender, smoking history, alcohol history, family history of malignant neoplasm, presenting symptoms, postoperative course, and previous diagnoses of pancreatitis and diabetes mellitus were evaluated for patients with IPMNs. Serum tumor markers including carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA) were recorded when available. Clinical data of 36 patients have been published previously (16).

Comparisons of the clinicopathological parameters were performed using the chi-square test or Fisher's exact test (for small numbers) for qualitative variables. Student's t test was used for quantitative variables and a non-parametric test (Mann-Whitney U test) if the distribution was abnormal. Significant predictive factors in the univariate analysis were then subjected to multivariate analysis. Multivariate analysis was performed by the logistic regression model, and results were expressed as the relative risk using a 95% confidence interval. Overall survival rates were calculated using the Kaplan-Meyer method, and univariate analysis was performed using the log-rank test. Survival was censored if the patient was still alive or had died from other causes. Statistical analysis was performed using Stat View (Version 5.0) software (Abacus Concepts, Berkeley, CA). All continuous data are presented as mean ± standard deviation of the mean. The presence of a statistically significant difference was denoted by $p<0.05$.

Duodenum-preserving Pancreatic Head Resection (DpPHR); Distal Pancreatectomy (DP)

RESULTS

Fifty-seven patients with IPMNs were managed surgically with curative intent from March 1991

TABLE 1 Analysis of Predictors for Malignancy and Invasive Carcinoma in 57 Patients with IPMNs

			Malignancy		Invasive IPMC	
Variables		**All**	**n**	***P***	**n**	***P***
Age	≤60	24	6	0.660	4	>0.999
	>60	33	10	5		
Sex	Male	41	8	0.054	4	0.109
	Female	16	8	5		
Smoking history	Yes	32	7	0.239	4	0.485
	No	25	9	5		
Alcohol history	Yes	30	8	0.804	5	>0.999
	No	27	8	4		
History of pancreatitis	Yes	10	6	0.022	2	0.651
	No	47	10	7		
Family history of malignancy	Yes	27	6	0.351	2	0.149
	No	30	10	7		
Diabetes	Yes	14	6	0.183	6	0.005
	No	43	10	3		
Symptomatic	Yes	25	12	0.003	6	0.161
	No	32	4	3		
Location	Head	39	12	0.504	7	0.704
	Body/Tail	18	4	2		
Tumor type	Main duct or combined type	13	8	0.004	5	0.022
	Branch duct type	44	8	4		
Mural nodule	Yes	36	16	0.0003	9	0.020
	No	21	0	0		
Serum CEA	Elevated	4	3	0.084	2	0.124
	Normal	44	12	6		
Serum CA 19-9	Elevated	9	4	0.352	4	0.040
	Normal	42	12	5		
Diameter of the tumor	≥30mm	21	6	0.724	4	0.434
	<30mm	29	7	3		
Diameter of the MPD	≥7mm	6	4	>0.999	2	>0.999
	<7mm	7	4	3		

through July 2004, and variables are summarized in **Table 1**. There were 40 male (70%) and 17 female (30%) patients, with ages ranging from 29 to 77 years (mean, 62 years) in this series. There were 32 (56%) current or former smokers, and 30 (53%) patients had a history of alcohol abuse. On the other hand, 10 (18%) patients had a history of pancreatitis, 27 (47%) had a family history of malignant neoplasm, 14 (25%) had diabetes mellitus, and 25 (44%) had symptoms on presentation. Thirty-nine (68%) of 57 patients had disease localized in the head of the pancreas. Seven IPMNs (12%) were of the main duct type, 44 (77%) were of the branch duct type, and 6 (11%) were of the combined type. The diameter of the MPD in the main duct type of IPMN ranged from 3 to 15mm, with a mean size of 8±4mm. The diameter of the cyst in the branch duct type ranged from 8 to 95mm, with a mean size of 32±18mm.

Operative procedures performed were as follows: pancreaticoduodenectomy (PD) (n=3), pylorus-preserving pancreaticoduodenectomy (PPPD) (n=16), pancreatic head resection with segmental duodenectomy (PHRSD) (n=16), segmental resection of the pancreatic body (SR) (n=12), duodenum-preserving pancreatic head resection (DPPHR) (n=1), distal pancreatectomy (DP) (n=7), and total pancreatectomy (TP) (n=2) **(Table 2)**. No operative or hospital deaths occurred.

Histological diagnosis was as follows: adenoma in 40 (70%) patients, borderline in 1 (2%), CIS in 7 (12%), and invasive carcinoma in 9 (16%). The mean follow-up period of all IPMNs was 61±35 months. The mean follow-up for the subgroups was as follows: benign IPMN (adenoma and borderline), 72 months (range 19 to 139 months); CIS, 26 months (range 7 to 47 months); and invasive IPMC, 38 months (range 1 to 90 months). We then analyzed the survival rates to assess postoperative prognosis of the 57 patients with IPMNs **(Figure 1)**. Patients with invasive IPMCs had a significantly shorter 3-year survival rate than patients with adenomas or CIS (80% *vs.* 100% *vs.* 100%; $p<0.0001$) in our series, when assessed by Kaplan-Meier curves.

The diameter of the tumor in the branch duct type of malignant IPMN was significantly larger than that observed in benign IPMN (**Table 3B**; 50.8±26.6 *vs.* 28.1±11.9; $p=0.008$, respectively). The diameter of the tumor in invasive IPMC was also significantly larger than that observed in non-invasive IPMN (54.8±28.7 *vs.* 30.0±14.9; $p=0.006$, respectively). The diameters of the MPD in the main duct type proved to be larger than those of the less malignant IPMN, however, the differences were not statistically significant.

We statistically analyzed factors predictive of malignancy and invasive carcinoma in patients with IPMNs. The 15 potential risk factors are listed in **Table 1**. Four factors were associated with malignancy by univariate analysis. The malignant group was more likely to be symptomatic, have a history of pancreatitis, present with the main duct or combined type, and contain a mural nodule. Significant predictive factors in the univariate analysis were then subjected to multivariate analysis. Among the factors analyzed, tumor type (main duct or the combined type) was an independent predictor of malignancy (**Table 4A**; risk ratio 5.47; $p=0.034$). The other four factors associated with invasive IPMC were as follows: previous diagnosis of diabetes mellitus, main duct or combined type, the presence of a mural nodule, and elevated serum CA 19-9 level. A previous diagnosis of diabetes mellitus and main duct or combined type tumor remained significantly associated with invasive IPMC on multivariate analysis (**Table 4B**; risk ratio 7.14, $p=0.042$ and risk ratio 4.07, $p=0.044$, respectively).

TABLE 2 Operative Procedures for IPMNs

	All	Benign IPMN	CIS	Invasive IPMC
PD	3	3	0	0
PPPD	16	12	0	4
PHRSD	16	10	4	2
SR	12	11	1	0
DPPHR	1	1	0	0
DP	7	4	1	2
TP	2	0	1	1
	57	41	7	9

DISCUSSION

Since the original description of IPMNs in 1982 (1), frequent reports have been published in the literature. Today, most gastroenterological surgeons

TABLE 3 Comparison of the Size in Each IPMN

(A) Comparison of the MPD Diameter in Main Duct or Combined Type of IPMN

	Benign IPMN (n=5)	IPMC (n=8)	*P* value
MPD diameter (mm)	7.1±4.3	8.8±5.2	0.549
	Non-invasive IPMN (n=8)	Invasive IPMC (n=5)	*P* value
	7.9±4.3	8.7±5.8	0.768

(B) Comparison of the Tumor Diameter in Branch Duct Type of IPMN

	Benign IPMN (n=36)	IPMC (n=8)	*P* value
Tumor diameter (mm)	28.1±11.9	50.8±26.6	0.008
	Non-invasive IPMN (n=40)	Invasive IPMC (n=4)	*P* value
	30.0±14.9	54.8±28.7	0.006

differentiate typical cases of IPMN from other mucinous or cystic tumors of the pancreas and understand the malignant potential of this neoplasm. The natural history of this disease and the factors that determine outcome in patients with this neoplasm, however, are not well understood (17). Furthermore, it is not easy to clearly distinguish between benign and malignant IPMN or non-invasive and invasive conditions with imaging tests (18-20). Preoperative or intraoperative evaluations for malignancy or invasion are needed for the appropriate management of IPMNs.

This neoplasm is often found in elderly men in the head of the pancreas (21). Age, gender, and location in our series of 57 patients showed a similar distribution. Patients with invasive IPMC had a poor prognosis and required more radical operations, for example PD or PPPD. Otherwise, non-invasive IPMN had a good prognosis, therefore, operations that preserve pancreatic or digestive function are preferable when preoperative examinations show no signs of invasion. One patient, who had undergone DPPHR, experienced ischemic necrosis of the common bile duct, and required reoperation. In order to prevent similar complications, we performed PHRSD for the low-grade malignant pancreatic head tumors after 1998 (22). We preserve the third portion and anal side of the second portion of the duodenum by conserving the gastroduodenal artery and the anterior inferior pancreaticoduodenal artery, and we resect the pancreatic head with 3 to 4cm of segmental duodenectomy including minor and major papilla. Several previous reports have demonstrated other less invasive surgical methods for IPMNs (23-26).

In this study, we analyzed factors predictive of malignancy and surgical outcomes in a large series. Our multivariate analyses demonstrated that the main duct and the combined type were independent predictive factors of malignant IPMN; in addition, both main duct or combined type and diabetes mellitus were independent predictors of invasive IPMC. However, the diameter of the MPD was not associated significantly with the extent of malignancy. Therefore, all IPMNs affecting the MPD required surgical treatment, irrespective of size. Recent reports have demonstrated that cancer was found in 60% of 140 patients with main duct type IPMNs (27). In the branch duct type of IPMNs, the tumor diameter correlated significantly with malignancy, therefore, larger tumors required more radical surgery. Tumor type and a history of diabetes are useful factors that must be preoperatively ascertained. Previous studies showed that p53 staining of resected specimen was a significant predictor of malignancy (28); however, p53 tumor expression is not available preoperatively to guide management.

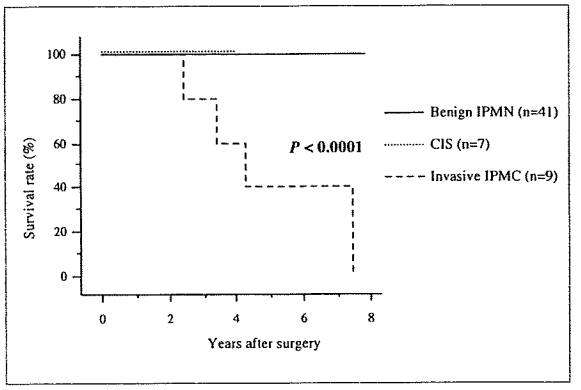

FIGURE 1 Kaplan-Meier survival curves show that patients with invasive IPMNs had a significantly shorter 3-year survival rate than patients with adenomas or carcinoma *in situ* (80% *vs.* 100% *vs.* 100%; $p<0.0001$).

We reviewed recent studies that investigated factors predictive of IPMN malignancy by statistical analysis (28-32). A MEDLINE search was conducted to identify articles in the English language that assessed the factors associated with IPMN malignancy from 1998 through 2004. The key words used included "intraductal papillary mucinous tumor (IPMT)," "intraductal papillary mucinous neoplasm (IPMN)," "predictive factor," and "predictor." Parameters associated significantly with IPMN malignancy are listed in **Table 5**. Our study demonstrated the presence of symptoms and a history of pancreatitis as significant factors, but other studies showed that these fac-

TABLE 4 Multivariate Analysis of Predictive Factors in Patients with IPMNs

(A) Factors predictive of malignancy	Risk ratio	95% Confidence Interval	*P* value
Tumor type (Main duct or combined type: Branch duct type)	5.47	1.14-26.27	0.034
Mural nodule (Yes: No)	7.46	0.74-75.68	0.089
Pancreatitis history (Yes: No)	3.48	0.61-19.96	0.161
Symptoms (Yes: No)	1.62	0.32-8.34	0.564
(B) Factors predictive of invasive IPMC	**Risk ratio**	**95% Confidence Interval**	***P* value**
Diabetes (Yes: No)	7.14	1.07-47.50	0.042
Tumor type (Main duct or combined type: Branch duct type)	4.07	1.65-25.51	0.044
Mural nodule (Yes: No)	5.71	0.48-67.18	0.166
Serum CA 19-9 (Elevated: Normal)	3.20	0.43-23.99	0.258